Analysis of the data set of the rate constants

The rate constants of folding (k_f) can be related to the Gibbs free energies of activation, $\Delta G_f^{0‡}$, using the transition state theory:

$$k_f = k^* \cdot \exp\left(-\frac{\Delta G_f^{0‡}}{RT} \right) \qquad (1)$$

The value of pre-exponential factor k^* represents the maximum rate for the folding reaction, which is assumed to be independent of both pressure (p) and the concentration of guanidine chloride ([GuHCl]). R is gas constant and T is temperature.

At constant temperature, $\Delta G_f^{0‡}$ can be also represented with the activation volume ($\Delta V_f^{0‡}$) as:

$$\mathrm{d}\,\Delta G_f^{0‡} = \Delta V_f^{0‡}\,\mathrm{d}\,p + m_f\,\mathrm{d}[\mathrm{GuHCl}] \qquad (2)$$

where the m_f value represents the change in $\Delta G^{0‡}$ with the [GuHCl] ($m_f = (\partial \Delta G_f^{0‡}/\partial[\mathrm{GuHCl}])_{p,T}$), which is empirically found to be constant over a wide range of GuHCl concentrations for protein folding reactions. To determine $\Delta G_f^{0‡}$ at a certain p and [GuHCl], the effects of pressure and GuHCl on the m_f value and on $\Delta V_f^{0‡}$ have to be taken into account. Because the differential of *eq.* (2) shows the following equation:

$$\frac{\partial^2 \Delta G_f^{0‡}}{\partial p \partial[\mathrm{GuHCl}]} = \left(\frac{m_f}{\partial p} \right)_{[\mathrm{GuHCl}]} = \left(\frac{\partial \Delta V_f^{0‡}}{\partial[\mathrm{GuHCl}]} \right)_p = n_f \qquad (3)$$

where n_f is the constant that is independent of both pressure and denaturant concentration, the integration of *eq.* (2) gives:

$$\ln k_f(p,[\mathrm{GuHCl}]) = \ln k_f(p_0,[\mathrm{GuHCl}]_0) - \frac{\Delta V_f^{0‡}([\mathrm{GuHCl}])}{RT} \cdot (p - p_0) - \frac{m_f(p_0)}{RT} \cdot [\mathrm{GuHCl}] \qquad (4)$$

$$\Delta V_f^{0‡}([\mathrm{GuHCl}]) = \Delta V_f^{0‡}([\mathrm{GuHCl}]_0) + n_f \cdot [\mathrm{GuHCl}] \qquad (5)$$

where $k_f(p,[\mathrm{GuHCl}])$ is the folding rate at p and [GuHCl] and $k_f(p_0,[\mathrm{GuHCl}]_0)$ is the folding rate at ambient pressure (0.1 MPa) and in the absence of GuHCl . $m_f(p_0)$ is the m_f value at ambient pressure. $\Delta V_f^{0‡}([\mathrm{GuHCl}]_0)$ is the activation volume in the absence of GuHCl.

The validity of the linear dependence of the activation volumes on the concentration of GuHCl can be assessed by *eq.* (3). Based on the pressure dependence of the m_f-values, the n_f-value was estimated to be 6.3 ± 2.4 $\mathrm{cm^3 \cdot mol^{-1} \cdot M^{-1}}$, while the GuHCl concentration dependence of the activation volumes showed that the n_f-value is 6.3 ± 2.0 $\mathrm{cm^3 \cdot mol^{-1} \cdot M^{-1}}$. Such coincidence in the n_f-values clearly indicates that the assumption of the linear fitting of the activation volume against the GuHCl concentration with *eq.* (5) is reasonable.

The data set of the rate constants determined at high pressure p and [GuHCl] can be, therefore, globally fitted with *eq*s. (4) and (5). The parameters; $k_f(p_0, [\mathrm{GuHCl}]_0)$, $m_f(p_0)$, and $\Delta V_f^{0‡}([\mathrm{GuHCl}]_0)$ were estimated to be

$11.9 \pm 0.9 \times 10^3\ s^{-1}$, $5.8 \pm 0.3\ kJ \cdot mol^{-1} \cdot M^{-1}$, and $-15 \pm 7\ cm^3 \cdot mol^{-1}$, respectively. By using the data set of the kinetic measurements at ambient pressure, $k_f(p_0, [GuHCl]_0)$ and $m_f(p_0)$ were also calculated to be $12.1 \pm 0.8 \times 10^3\ s^{-1}$ and $5.3 \pm 0.4\ kJ \cdot mol^{-1} \cdot M^{-1}$, respectively, both of which are virtually the same as those from the high pressure measurements (10-200 MPa) ($11.9 \pm 0.9 \times 10^3\ s^{-1}$ and $5.8 \pm 0.3\ kJ \cdot mol^{-1} \cdot M^{-1}$, respectively). The good agreement of kinetic parameters at ambient and high pressure demonstrates the reliability of the estimation of the activation volume in the absence of the denaturant at ambient pressure.